Docket No. MWH-0029US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Stephen B. Liggett
Application No.:	09/856,803
Filed:	May 25, 2001 (35 U.S.C. § 371 of PCT/US99/27963, filed November 24, 1999, which claims benefit of U.S. Appl. No. 60/109,886, filed November 25, 1998)
Confirmation No.:	3706
Group No.:	1634
Examiner:	Myers, C.
For:	**POLYMORPHISMS IN THE 5' LEADER CISTRON OF THE β_2-ADRENERGIC RECEPTOR**

Commissioner for Patents
Washington, D.C. 20231

Certificate of Facsimile Transmission
I hereby certify under 37 C.F.R. § 1.8 that this correspondence is being transmitted by facsimile to the United States Patent and Trademark Office, Commissioner for Patents, TC 1600, at (703) 872-9306, on May 7th, 2003

Matthew M. Catlett

DECLARATION OF STEPHEN B. LIGGETT, M.D., UNDER 37 C.F.R. § 1.131

This Declaration Of Stephen B. Liggett, M.D., Under 37 C.F.R. § 1.131 is being submitted as part of Applicant's Response To Office Action Under 37 C.F.R. § 1.11 regarding the office action dated January 7, 2003 that was received in the captioned application.

Being warned that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements and the like may jeopardize the validity of the instant application or patent resulting therefrom, I hereby declare that:

1) I am the original, sole, and first inventor of the subject matter that is claimed in pending claims 1-8 and 11 of the captioned application, namely (a) a method for genotyping the

β_2-adrenergic receptor (β_2AR) gene of an individual comprising determining the identity of the nucleotide pair at the 5' leader cistron (5'LC) polymorphic site (PS), which, as is demonstrated throughout the specification of the captioned application, is located 47 bases upstream of the β_2AR coding region, which begins at nucleotide position 1588 of SEQ ID NO:1 (thus, the 5'LC PS is located at nucleotide position 1541 of SEQ ID NO:1) in the two copies of the β_2AR gene present in the individual; and (b) a method for genotyping the β_2AR gene of an individual comprising determining the identity of the nucleotide pair at the 5'LC PS and at one or more additional PSs in the β_2AR gene in the two copies of the β_2AR gene present in the individual.

2) Further to an effort, dating back to as early as January of 1996 (see attached copies of PCR protocols), to discover polymorphisms in the region upstream of the β_2AR gene, I directed the performance of an experiment designed to elucidate the existence, if any, of such polymorphisms. Utilizing PCR techniques to analyze genomic DNA in this region from human volunteers, I discovered, in the "sense" strand, the existence of a thymine residue 47 bases upstream of the β_2AR coding region, as well as the existence of an adenine residue 47 bases upstream of the β_2AR coding region in the "antisense" strand. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-1369 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region; chromatogram #096-1364 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region; chromatogram #096-1367 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region; and chromatogram #096-1362 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region). Although all previous reports indicated that the only known residue at the nucleotide position located 47 bases upstream of the β_2AR

coding region, in the "sense" strand, was a cytosine (and thus, in the "antisense" strand, a guanine), to confirm that I had indeed discovered a polymorphism at this position, I subsequently directed the performance of a similar experiment with the wild-type sequence, and discovered, in the "sense" strand, a cytosine, and in the "antisense" strand, a guanine. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-2859 demonstrates a cytosine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region; and chromatogram #096-2860 demonstrates a guanine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2AR coding region). My discovery of this polymorphism, and my subsequent confirmation of this discovery, occurred prior to the effective date of any of the following references: Timmermann *et al.*, *Kidney Intl.* 53:1455-60 (June 1998), Timmerman *et al.*, *J. Molecular Med.* 76:B30, Abst. P-109 (May 1998), Timmermann *et al.*, *Human Mutation* 11(4):343-4 (March 1998). With respect to the copies of the chromatograms, the nucleotide position that is 47 bases upstream of the β_2AR coding region is that denoted with a "^" symbol.

3) All statements made herein of my knowledge are true, and all statements made herein on information and belief are believed by me to be true.

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Applied Biosystems
Model 373A
Version 2.0.1S

B579 20
DyeTerminator{AnyPrimer}
Lane 20
Signal: G:757 A:1184 T:300 C:377

INST FILE 808296
096-1369
MCGRAW--A-2/F-1

Tue, Mar 5, 1996 8:51 PM
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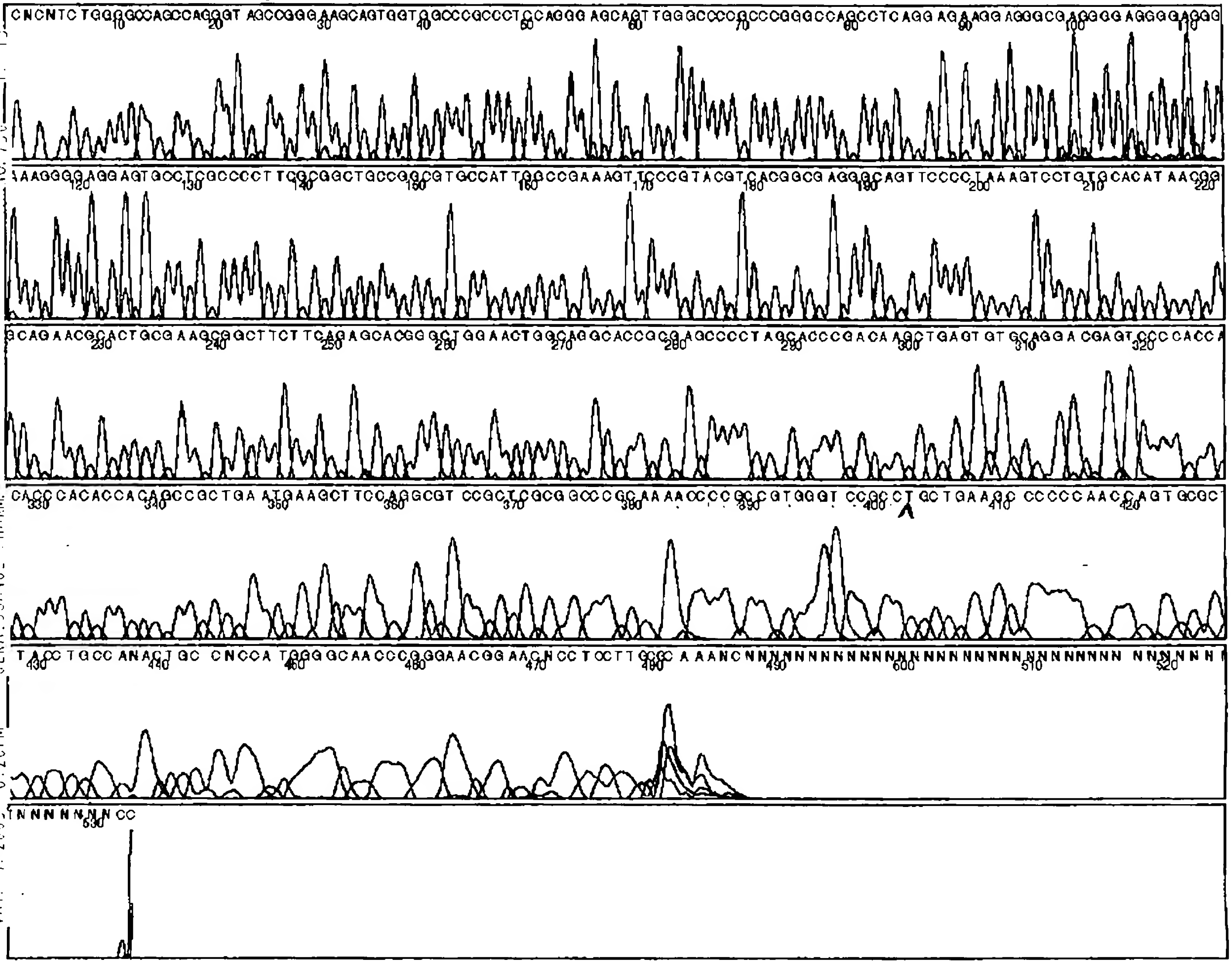
A2-F



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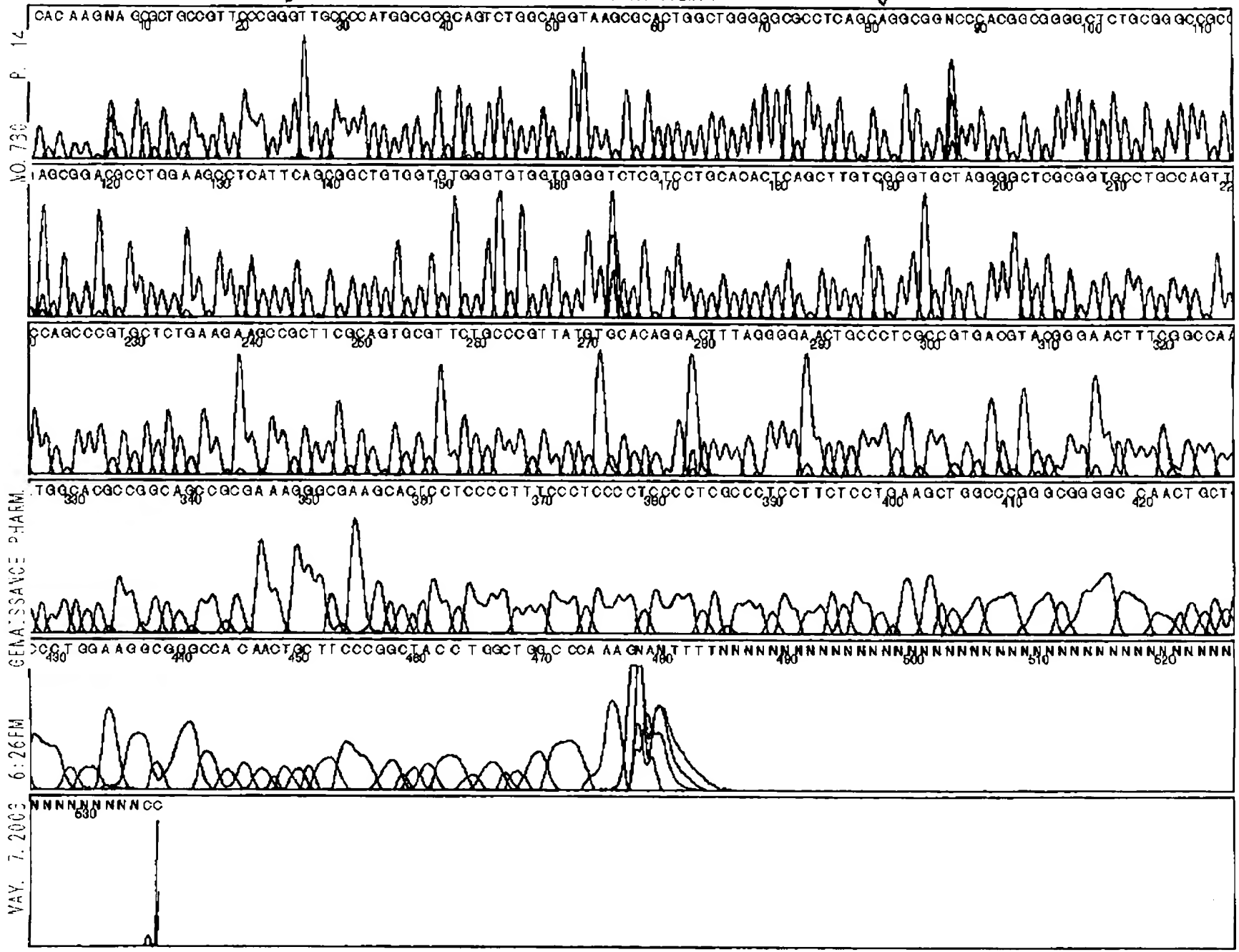

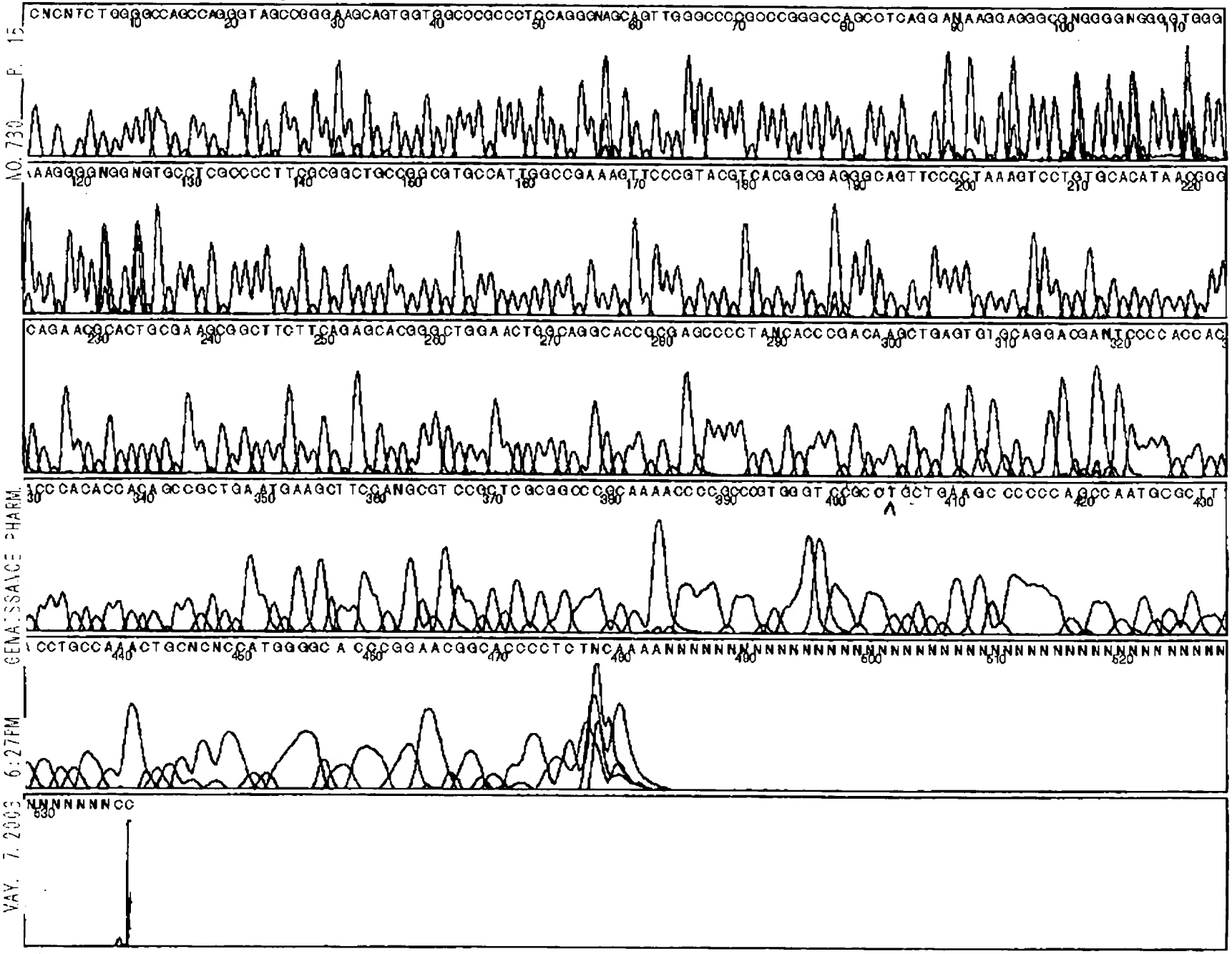

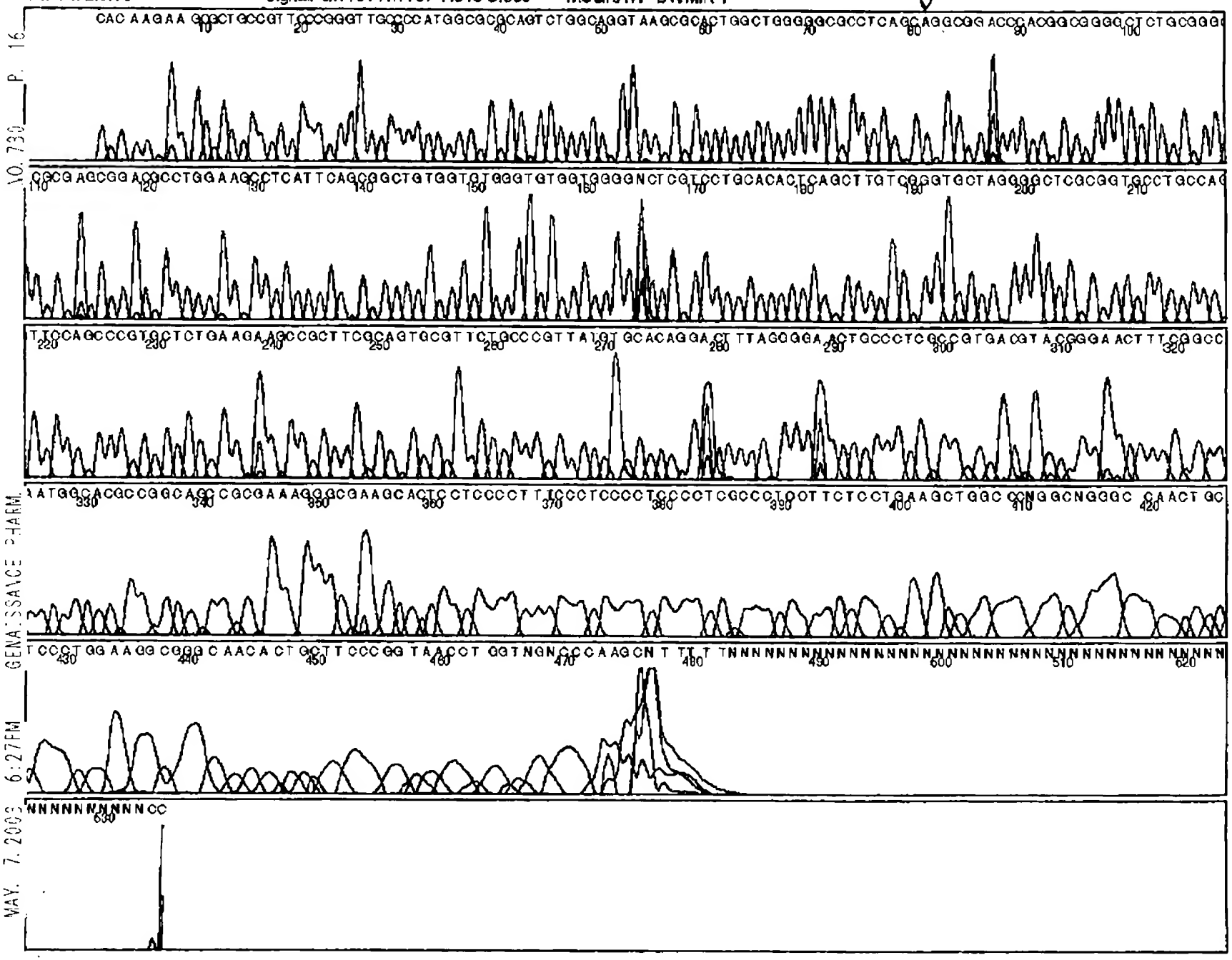

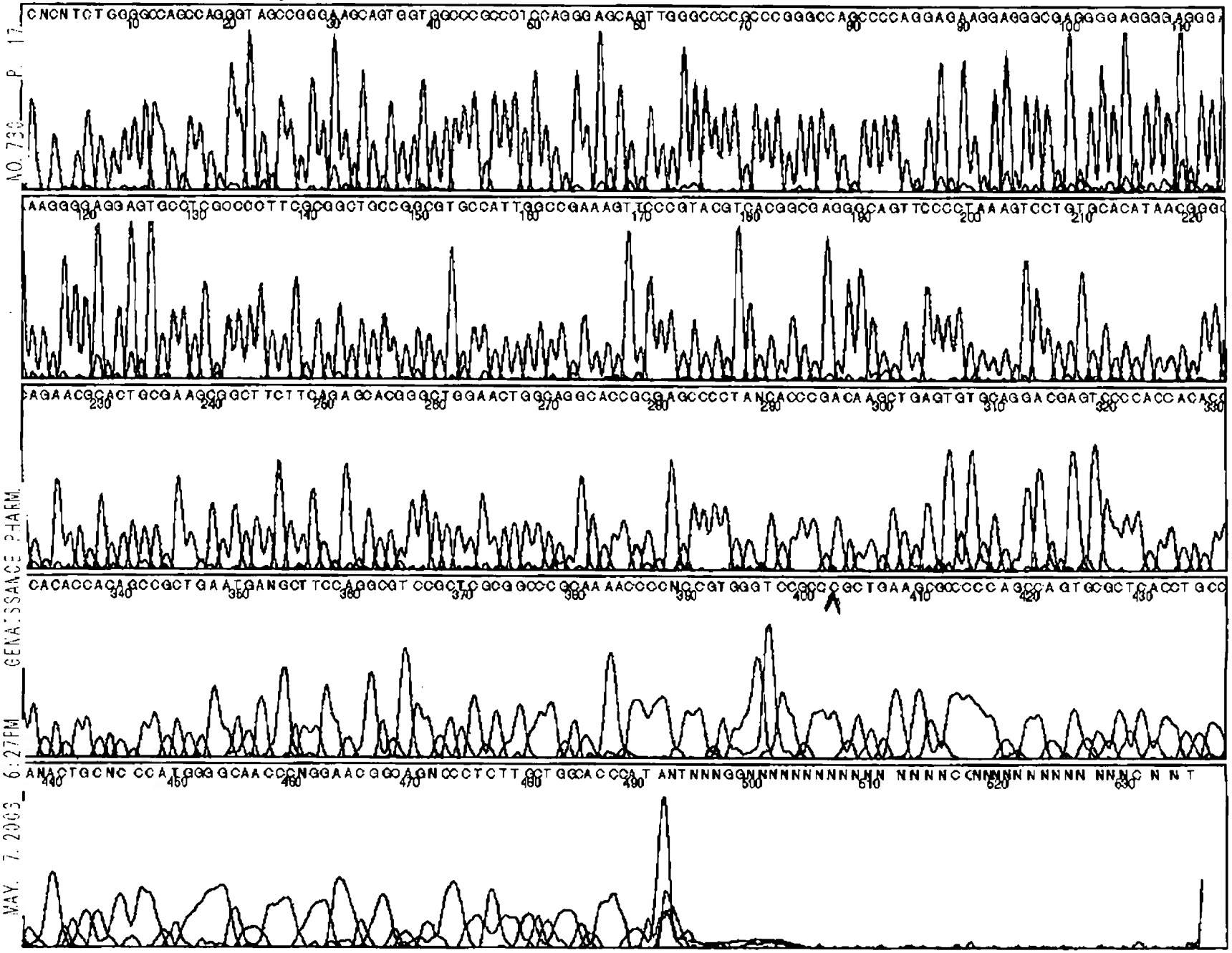

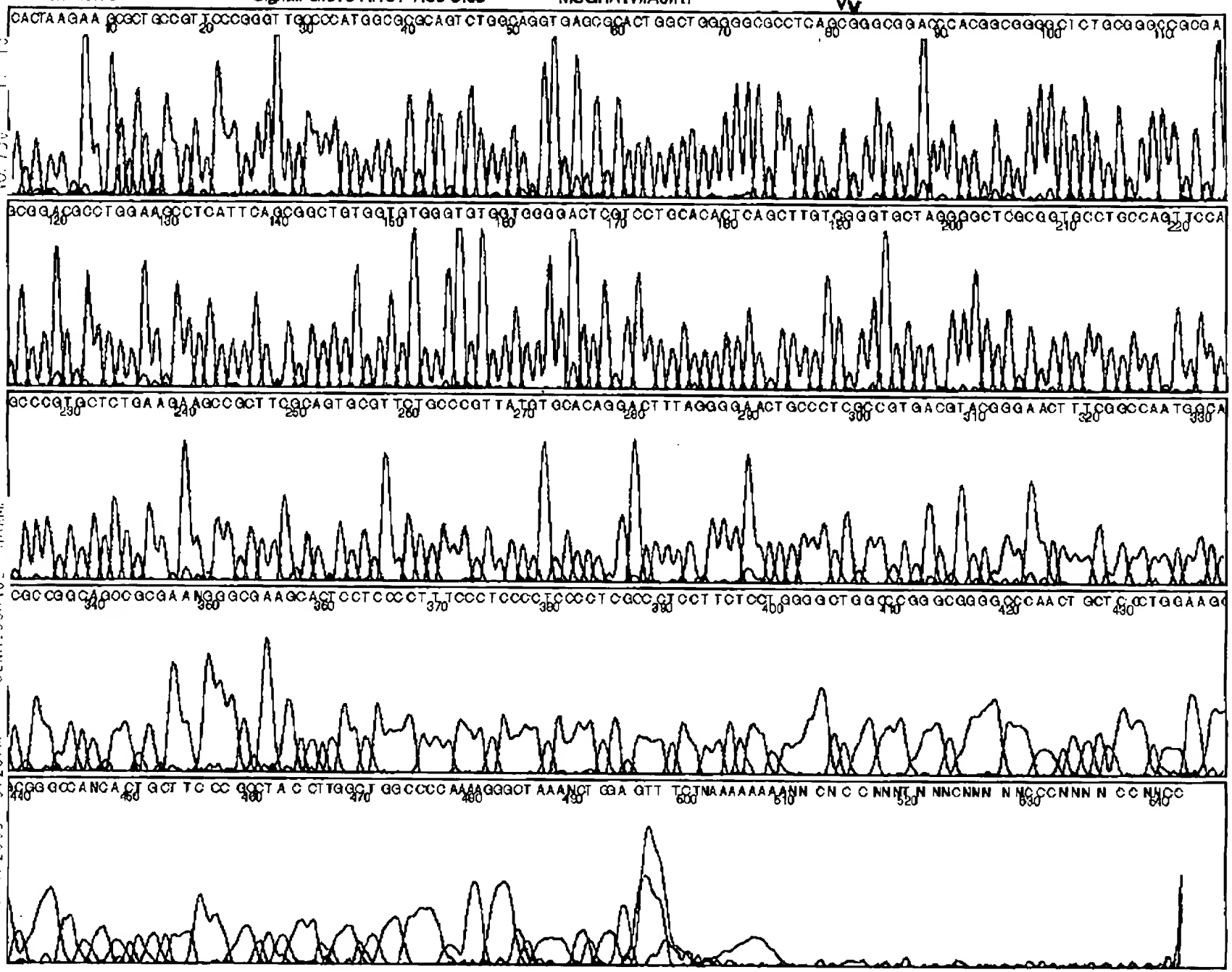

PCR (β_2AR) 1/26/96

(* Made new set of primers for β_2AR 5' flanking region. Product should include the CRE + short ORF. Primers were chosen in MacVector

Blood samples are being obtained from pts in the UC asthma clinic by Melanie Meyers.

DNA samples prepared by Tim Donnelly using Invitrogen kit/or Qiagen kits. Samples are numbered as received (A1, A2, etc.)

- Set up PCR rxn w/ new primers. Made up 96 well master mix:
  - 10 µl buffer II
  - 6 µl 25mM $MgCl_2$
  - 0.8 µl 25mM dNTP
  - 0.5 µl 100µM forward primer (β_2AR-F1)
  - 0.5 µl 100µM reverse primer (β_2AR-R1)
  - 77.7 µl dH_2O
  - 0.5 µl enzyme
- aliquot 24 µl of master mix into PCR tube
- add 1 µl template DNA
- overlay w/ 1 drop mineral oil
- perform PCR in thermocycler (stratagene robocycler) w/ temp gradient mode as
  - 94°C x 2 min
  - 94°C x 30 sec / 64°, 62°, 60°, 58°C x 30 sec / 72°C x 30 sec => 35 cycles
  - 72°C x 7 min
- run 10 µl of per rxn on TBE minigel

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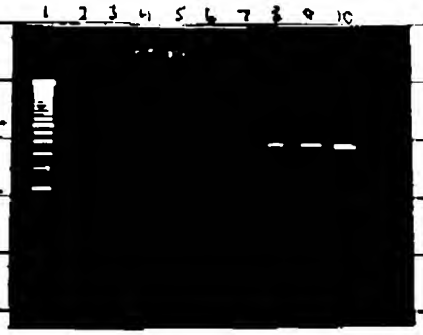
(cont) for primer info

1/26/96 (cont)

see GAK RIA section 1 original plan (1/26/96)



1 - 100 bp ladder		6 - blank	
2 - A1 (Invitrogen)	64°C	7 - A1 (Qiagen)	64°C
3 - "	62°C	8 - "	62°C
4 - "	60°C	9 - "	60°C
5 "	58°C	10 - "	58°C

No product seen ± DNA isolated by Invitrogen kit. Nice band seen ± DNA from Qiagen kit but expected size should be 538 bp. The band present appears to be <400 bp.

Repeat PCR with following modification:

- add 73.7 µl dH_2O
- dispense 23 µl of master mix into PCR tubes
- add 2 µl template DNA
- change annealing temp for Invitrogen template to 56°, 54°, 52°, 50°C; change annealing temp for Qiagen template to 58°, 56°, 54°, 52°C

1 - 100 bp ladder		6 - 100 bp ladder	
2 - A1 (Invitrogen)	56°C	7 - A1 (Qiagen)	58°C
3 - "	54°C	8 - "	56°C
4 - "	52°C	9 - "	54°C
5 - "	50°C	10 - "	52°C

Now have band in all samples. However, apparent band present in both samples size appears to be <500 bp, still smaller than expected.

5/1/96

β₂HR PCR (5 kb)

Set up master mix for four (4) 25 µl PCR rxns:

2 µl	template DNA (DW m)
1.5 µl	forward primer
1.5 µl	reverse primer
20 µl	5X buffer (buffer N from Stratagene PCR optimizer kit)
10 µl	dNTPs (2.5 mM) (from optimizer kit)
65 µl	dH_2O
0.8 µl	Taq
100 µl	total

- aliquot 25 µl of master mix into 4 PCR rxn tubes
- overlay ~ ½ drop of mineral oil
- PCR cycle

98°C	2 min	
98°C	30 sec	} 30 cycles
56°, 54°, 52°, 50°	30 sec	} 30 cycles
72°C	30 sec	} 30 cycles
72°C	7 min	

- remove 10 µl aliquot + run on minigel

#1 - 100 bp ladder
#2 - 56°C
#3 - 54°C
#4 - 52°C
#5 - 50°C